Solvent Stokes-Einstein violation in aqueous protein solutions

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The Stokes-Einstein equation is applied to the water self-diffusion coefficient D in human serum albumin protein solutions. A linear trend for D as a function of T/η is found for all the protein concentrations investigated. However, the indication of a violation of the Stokes-Einstein equation is found in the protein concentration dependence of the effective hydrodynamic radius of water. The deviation of the experimental NMR water self-diffusion and viscosity data from the hydrodynamic Stokes-Einstein relation is found to be consistent with an enhancement of the solvent structure in the vicinity of the protein surface.

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INTRODUCTION

The diffusion coefficient D of a particle moving in a fluid is in a strict connection with its shear viscosity η. For many fluids this relationship is expressed by the Stokes-Einstein formula

$$D=\frac{kT}{C\eta r}\ , \tag{1}$$

where r is the radius of the diffusing particle and C is a constant depending on the theoretical arguments used for the derivation of Eq. (1) [1–6].

In the most well-known approach, that of Einstein, the hydrodynamic Stokes formula for the viscous drag force on a moving sphere is used and the constant C assumes a value which ranges from 6π, under no-slip boundary conditions, to 4π, under slip boundary conditions for the fluid on the particle surface [1].

This approach should, in principle, only apply to diffusing particles that are much larger than the molecules comprising the fluid. However, the Stokes-Einstein formula works also in the case of small diffusing particles and self-diffusion in many liquids. Moreover, some theoretical derivations of Eq. (1), which extend its validity to both these cases, exist [2,3]. They start from the Green-Kubo formulas, which relate D and η to the autocorrelation functions of the velocities and forces, respectively, and evaluate the constant C, which now assumes different values and meanings with respect to the hydrodynamic approach. In particular, the constant C results in being dependent on the structure of the fluid and then on the relative interactions among its particles.

It is often used to define an effective hydrodynamic radius r^* so that Eq. (1) is rewritten in the hydrodynamic form with no-slip boundary conditions as

$$D=\frac{kT}{6\pi\eta r^*}\ , \tag{2}$$

where now the difference of C from 6π is included in r^*, which of course does not represent the real size of the diffusing particle anymore, but contains information about the fluid structure.

If Eq. (1) is satisfied, the effective hydrodynamic radius r^*, is, of course, constant, even if some fluids exist (for example, some glass-forming liquids [6]) in which r^* is temperature dependent and then violate the Stokes-Einstein equation.

In this work we focus attention on the solvent self-diffusion and viscosity of water-protein solutions. We present experimental data of water D and solution viscosity in the temperature range from 0 to 40 °C for two protein concentrations. We find an effective hydrodynamic radius of water, which results in being dependent on the protein concentration and in this sense violates the Stokes-Einstein equation. The experimental results are interpreted in terms of an enhancement of the solvent structuration in the vicinity of the protein surface.

EXPERIMENT

Aqueous protein samples were prepared by dilution in a phosphate buffer of dry human serum albumin (HSA) powder (fatty acid free), purchased from Sigma Chem. Co., and used without further purification. HSA is a globular protein of 69 000 daltons with a stable tertiary structure for temperatures lower than 70 °C, at which denaturation occurs; in particular, in the temperature range investigated, no significant structure changes are observed by circular dichroism. The buffer was composed of KH_2PO_4 and Na_2HPO_4 and had a pH of 6.96 at 20 °C and a total concentration of 4.24×10^{-2} M. The protein concentration of each sample was determined by weight and the pH of the most concentrated solution was 6.99 at 20 °C.

Water self-diffusion measurements were performed on

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a home-built 10-MHz low-resolution NMR spectrometer [7] with the static magnetic field gradient technique [8] by comparing the zero gradient spin-echo amplitude with the amplitude of the echo at different field gradient values. The field gradient was calibrated using pure water at 25 °C as a reference [9]. Temperature control was performed by inserting the sample into a nitrogen flux at a predetermined temperature, which was made constant by an on-off heating controller. Spin-echo curves, as a function of the square of the gradient intensity, were fitted by a single exponential least squares fit routine. The observation time of the diffusion process, i.e., the interval in which the molecules are exposed to the gradient field, was taken as constant (36 ms) in the overall temperature range.

The solution's shear viscosity coefficients were obtained by the Ostwald method, measuring the flow time of a fixed volume of liquid under the gravitational force. The temperature was controlled by taking the viscosimeter immersed in a thermal bath. Water at 25 °C was used as a reference [10]. The mass density of the two protein solutions, as a function of the temperature, was calculated from the water density by assuming a specific volume for the protein of 0.74 $cm^3\ g^{-1}$ [11].

RESULTS AND DISCUSSION

Figure 1 shows the water self-diffusion coefficient as a function of the ratio between the temperature and the solution viscosity for the solvent buffer and for two different protein concentrations. The trend of the experimental data is linear in agreement with Eq. (1), but the increment of the line slope with the protein concentration is indicative of a decreasing in the hydrodynamic radius of water (see the figure inset). This substantial violation of the Stokes-Einstein formula can be understood by considering a strong contribution of the solute-solute interaction to the solution viscosity. In fact, many terms contribute to the viscosity of the solution: the molecular size of both the components, and the solvent-solvent, solvent-solute, and solute-solute interactions. In addition, the presence of a solute particle should also locally modify the strength and the shape of the interaction among solvent molecules [4,6,7,12]. On the other hand, the solute-solute interaction does not contribute to the self-diffusion coefficient of the solvent molecules, whereas the size of the solute molecules contributes only indirectly through the modification of the solvent structure. The fact that the interaction among solute particles contributes only to the solution shear viscosity and not to the solvent self-diffusion has the obvious effect of reducing the apparent hydrodynamic radius of the water molecules. This is not a surprising result, since the diffusing water molecules essentially sense a local viscosity, due to the surrounding solvent molecules, which should be different from that of the overall solution in which the direct interaction among the solute particles may play a significant role.

If the presence of the solute does not influence the structure of the solvent, then the viscous force which a diffusing water particle experiences is that of a pure solvent. In Fig. 2, we show the water self-diffusion coefficient measured in different protein solutions as a function of T/η_{buffer}, where η_{buffer} is the buffer shear viscosity coefficient. Also in this case a linear behavior is observed in agreement with Eq. (1), but with a different trend for the line slope when compared to that shown in Fig. 1. The hydrodynamic radii obtained from Eq. (2) are shown in the inset of Fig. 2, where an increment of the apparent hydrodynamic radius of water with the protein concentration is observed. Since in this case no contribution from the solute-solute interaction is expected, the

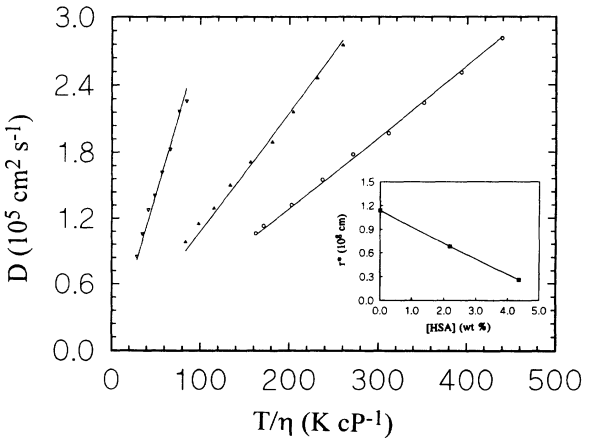

FIG. 1. Self-diffusion coefficient of water vs the ratio between the temperature and the solution shear viscosity of (i) water buffer (open circle), (ii) HSA 2.8% (gram of protein per gram water) (solid triangle), and (iii) HSA 4.3% (gram of protein per gram water) (open triangle). The solid line represents the linear best fit. In the inset the effective hydrodynamic radius of water, calculated from Eq. (2), is plotted (solid squares) as a function of the protein concentration (the continuous line is only a visual guide).

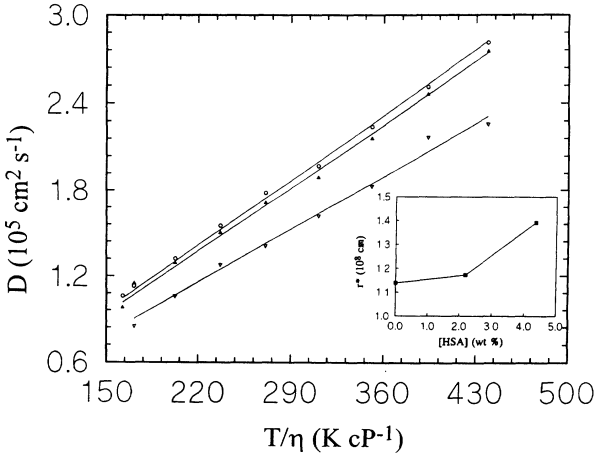

FIG. 2. Self-diffusion coefficient of water vs the ratio between the temperature and the buffer shear viscosity of (i) water buffer (open circle), (ii) HSA 2.8% (gram of protein per gram water) (solid triangle), and (iii) HSA 4.3% (gram of protein per gram water) (open triangle). The solid line represents the linear best fit. In the inset the effective hydrodynamic radius of water, calculated from Eq. (2), is plotted (solid squares) as a function of the protein concentration (the continuous line is only a visual guide).

violation of the Stokes-Einstein relation as a function of the protein concentration should be completely ascribed to the changes of the solvent structure as due to the presence of the protein solute.

Very recently violations of the Stokes-Einstein equation in glass-forming liquids have been interpreted in terms of a smooth space variation of the viscosity near a spherical particle. In that work the authors assume an exponential dependence of the viscosity on the distance from the particle surface and evaluate the drag force by solving the appropriate Navier-Stokes equation [6]. They assume a lower value of the viscosity near the protein surface with respect to the bulk, and this results in a reduction of the drag force. Conversely, if the fluid near the surface is more viscous than the bulk, the drag force, and then r^*, should increase.

We have recently connected the self-diffusion properties of water in protein solutions to an enhancement of the hydrogen bonding near the biomolecule surface, which decays exponentially with the distance from the protein molecule [7,12]. By assuming an overlap between the hydrogen bond forming probability functions associated with different biomolecules when the protein concentration is increased, we were able to accurately reproduce the experimental data. In addition, such an increment of the H bonding in the protein solution was observed by near infrared spectroscopy [13]. We could now argue that the violation of the Stokes-Einstein equation shown in Fig. 2 can be connected to a local increment of the shear viscosity near the protein surface as due to the H-bond probability enhancement induced by the structural and motional constraints applied by the biomolecule to the solvent H-bond network [7,14]. In fact, biomolecules move much more slowly than water molecules, and this makes them apply motional constraints to the solvent molecules. These constraints are expected to propagate along pathways of two H-bonded water molecules and to be reinforced in clusters and patches of four H-bonded water molecules. Therefore, near the biomolecule an increment of the probability of finding water clusters with high density of formed H bonds is expected [7,12]. This increment of the water structure near the protein surface could be responsible for the local increment of the solvent viscosity with respect to the bulk and then could explain the increment of the effective hydrodynamic radius of water [6]. Moreover, by increasing the protein concentration, an even more significant structuration of the solvent water is expected, due to the overlap of the protein induced H-bond probabilities [7,12]; thus the effective hydrodynamic radius r^* should become concentration dependent. On the other hand, the possibility that the apparent increasing of r^* could be due to obstruction effects cannot be ruled out. In fact, the presence of large and slow moving protein molecules could force the diffusing water molecules to follow longer paths than in the case of free diffusion, with a consequent reduction of D, without any change in the viscosity [15,16]. However, the interpretation of experimental diffusion data as a function of the protein concentration by the obstruction model would require the assumption of higher hydration water fractions than those experimentally determined [12,16,17], indicating that such an effect might only partially be responsible for the decrease of D.

In conclusion, in the present work we have put into evidence the violation of the Stokes-Einstein relation in the self-diffusion of water in the presence of globular proteins. We believe that this violation is mainly due to a long range water structuration propagating from the protein surface, even if some contribution from a geometric blocking effect on water diffusion by the large protein molecules cannot be ruled out.

Finally, it is worthwhile to remark that the linear behavior of D versus T/η in both Figs. 1 and 2 implies a practical temperature independence of the viscosity near the protein surface, which is, however, different from that of the bulk. However, this situation could not necessarily persist in the supercooled region where the strong cooperative behavior of the H-bond network should play a very crucial role and the formation of a high connected H-bond structure is favored [18]; in that case, a behavior similar to that observed in some glass-forming liquids could be observed [6].

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